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Insolubilization Process Increases Enzyme Stability

Enzymes rendered insoluble by attachment to polymeric matrices have properties which suggest novel application to enzyme-controlled reactions. The modified enzymes may have properties that are significantly different from those of the original enzymes; in particular, the stability of several modified enzymes is greater. Moreover, the physical form of the insolubilized enzymes makes them useful in column processes where the substrate is passed through a bed; in batch processes, the enzymes can easily be recovered for reuse. More importantly, it is possible to exercise control over enzymatic functions by the use of appropriate polymeric matrices, because the pH optima and K_m (equilibrium constant) values of insolubilized enzymes are determined by the type of polymers to which they are attached.

In recent years, a large number of enzymes have been complexed with polymeric matrices by a variety of synthetic techniques. Almost exclusively, however, only low molecular weight, hydrolytic enzymes of relatively simple structure have been modified by these techniques. It has been difficult to synthesize polymer adducts with enzymes of complex structure, particularly the enzymes of the Calvin cycle, which are sensitive to chemical reagents.

Three of the large-molecule enzymes of the Calvin cycle have been successfully incorporated into polymeric matrices by the optimization of synthetic techniques. Extension of these techniques to other enzymes of the Calvin cycle should lead to the structuring of enzyme-packed columns capable of converting metabolic wastes into edible products; e.g., carbon dioxide into fructose or glucose.

Experimental results indicate that large enzyme molecules such as aldolase, glyceraldehyde-3-phos-

phate dehydrogenase (GAPD), and fructose-1,6-diphosphatase (FDP-ase) may be attached to polymeric matrices and that the enzymes retain a portion of their catalytic activities. Three different carrier materials were used to form these enzyme-polymer adducts: ethylene maleic anhydride (EMA), p-aminobenzyl cellulose (PAB), and aminoethyl cellulose (AEC).

The stability of insolubilized enzyme derivatives is markedly greater than that of soluble enzymes. For example, aldolase-EMA in either powder or suspension form was found to be stable for a period of 20 days at 4° C; in contrast, soluble aldolase lost more than 50% of its activity in 15 days. A suspension of aldolase-AEC was found to be stable over a period of 21 days. The soluble form of GAPD became completely deactivated overnight; in contrast, the GAPD-AEC retained most of its enzymatic activity after 1 day.

Studies of the range of optimum pH values revealed that the enzyme-polymer adducts were significantly different from their progenitors. In general, the attachment of enzymes to basic polymers resulted in shifts of optimal pH to lower values, and the attachment to acidic polymers resulted in shifts to higher values. For instance, soluble aldolase exhibited optimum activity at pH 8, but the modified forms were different: aldolase-PAB, pH 6.0; aldolase-AEC, pH 6.5; and aldolase-EMA, pH 9.0. Soluble GAPD exhibited optimum activity at pH 9.5, but the GAPD-AEC exhibited an optimum at pH 7.0. The optimum of soluble FDP-ase, pH 9.2, was shifted to pH 8.5 when attached to AEC.

Studies of the reaction between the insolubilized adducts and their substrates demonstrated that the K_m value was determined by the type of polymer

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to which the enzyme was attached. When the enzyme was attached to basic polymers, the K_m values were lower than those of the soluble enzymes. Conversely, the K_m values of the enzyme-acidic polymer adducts were higher than those of the soluble enzymes.

The insolubilized enzyme adducts formed between aminoethyl cellulose and each of the three enzymes contained the largest amounts of protein and retained most of the original enzyme activities. These adducts were remarkably stable at room temperature. A sequential reaction using columns of aldolase and GAPD was demonstrated. The substrate for the aldolase column was fed at the top, and the eluate became the substrate for the GAPD column. The result of the reaction was visually demonstrated by the disappearance of the blue color of methylene in the presence of diaphorase activity.

Notes:

1. The following documentation may be obtained from:

National Technical Information Service
Springfield, Virginia 22151
Single document price \$3.00
(or microfiche \$0.95)

Reference:

NASA CR-73354 (N70-23428), Studies on the Optimization of Techniques for Enzyme Insolubilization.

2. Requests for further information may be directed to:

Technology Utilization Officer
Ames Research Center
Moffett Field, California 94035
Reference: B71-10443

Patent status:

No patent action is contemplated by NASA.

Source: J. Billingham
Ames Research Center, and
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Battelle Memorial Institute
under contract to
Ames Research Center
(ARC-10314)